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Aquaculture 243 (2005) 291–303

Aquaculture

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Use of commercial *Artemia* replacement diets in culturing larval American lobsters (*Homarus americanus*)

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Received 16 June 2004; received in revised form 11 October 2004; accepted 12 October 2004

Abstract

This work reports on the successful incorporation of commercial formulated *Artemia* replacement diets as 50% of a larval American lobster diet. Combination diets of either live *Artemia* nauplii or frozen adult n-3 fatty acid enriched *Artemia* with a rotation of three commercial formulated diets resulted in equivalent survival to stage IV (19–25%), postlarval size and subsequent early juvenile performance compared to an *Artemia* nauplii plus frozen *Artemia* combination diet. A 100% formulated diet resulted in reduced larval survival (6%) and postlarval size, while a larval diet of 100% frozen adult *Artemia* resulted in reduced postlarval quality and early juvenile performance. The much lower price of the formulated diets compared to the prices of *Artemia* nauplii and frozen *Artemia* makes its inclusion in the lobster larval diet the most cost-effective diet choice.

Keywords: *Artemia*; Artificial feed; Captive rearing; Cost/benefit ratio; *Homarus americanus*; Larval diet

1. Introduction

Homarid lobster (*Homarus americanus* and *H. gammarus*) larviculture has historically relied upon the exclusive use of live and/or fresh-frozen diets. Lobster larvae are primarily carnivorous, their natural diet being composed of copepods, other zooplankton and to a lesser degree phytoplankton (Harding et al., 1983; Juinio and Cobb, 1992; Ennis, 1995). They are capable of capturing and consuming prey within hours of hatching (Biesiot and Capuzzo, 1990) and, as development progresses, they become increasingly voracious with a preference for larger prey. Like other carnivorous crustacean larvae, lobster larvae are cannibalistic in communal culture (Wickins and Lee, 2002). Lobsters have three planktonic larval stages during which communal culture is necessary, before metamorphosis to the benthic postlarval stage occurs and they can be transferred to individual containers. If the diet provided is inadequate or unpalatable, cannibalism can be extensive, resulting in major losses.

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doi:10.1016/j.aquaculture.2004.10.009

Commercial lobster aquaculture has been extremely limited, in part because of the high cost and inconvenience of using natural diets (Conklin, 1995; Nicosia and Lavalli, 1999). In recent decades, both large and small-scale lobster hatcheries have primarily relied on *Artemia* in larval diets (Table 1). Live adult *Artemia* has been found to be an excellent diet for lobster larvae, resulting in a high percentage of larvae surviving to metamorphose to healthy postlarvae (Conklin, 1995), while frozen adult *Artemia* or live *Artemia* nauplii each support lower levels of survival. Unfortunately, live adult *Artemia* is excessively expensive to purchase, and labor intensive to produce. For these reasons, frozen adult *Artemia* and/or freshly hatched *Artemia* nauplii continue to be used in the larval lobster diet, both in research laboratories and larger scale hatcheries.

It is difficult to assess the historical effort placed on finding suitable artificial homarid larval diets. Whereas it had undoubtedly been attempted previously, such efforts were probably underreported due to a lack of positive results. Chang and Conklin (1993) list "artificial preparations" as one of a variety of foodstuffs that lobster larvae will consume in the laboratory, then go on to state that they used live adult *Artemia* almost exclusively in their facility, occasionally substituting frozen *Artemia*. Kurmaly et al. (1990) succeeded in rearing European lobster (*H. gammarus*) larvae to stage III on formulated diets, but because the larvae were held individually, cannibalism was not an issue. To date, no diet formulations have been developed specifically for lobster larvae. In contrast, there are now a number of formulated diets available for larval and postlarval penaeid shrimp. Postlarval penaeid shrimp, like larval lobsters, are ideally reared on live *Artemia* and are cannibalistic when the diet provided is unpalatable or insufficient (Wickins and Lee, 2002). The growth of the shrimp aquaculture industry has resulted in a high demand for shrimp diets and feed manufacturers have responded by investing effort into developing formulated *Artemia* replacement diets. These diets are now successfully being used to partially replace live *Artemia* in commercial shrimp hatcheries. Because of the significantly lower costs of these diets compared to *Artemia*, even partially replacing *Artemia* in the diet can result in considerable savings.

This work evaluated the feasibility and cost effectiveness of communally rearing lobster larvae on commercial *Artemia* replacement (CAR) diets. Larval American lobsters (*H. americanus*) were reared in experimental kreisels on a live *Artemia* nauplii diet, a frozen adult *Artemia* diet, a rotation of three CAR diets or on paired combinations of these diets. Diet combinations were tested using a three way factorial design, over the course of two trials. Growth, survival, postlarval quality and development rates were evaluated, and benefit–cost

Table 1
Larval diets used in lobster hatcheries

Diet	Species	% Survival	Location	Citations
Frozen mysids (*Neomysis* sp.) +live *Artemia* nauplii	*H. gammarus*	Variable, means <10%–34%	Conwy, UK	Wickins, 1998; Beard and Wickins, 1992
Live *Artemia* nauplii+ phytoplankton	*H. gammarus*	47%	Carna, Ireland	Browne and Mercer, 1998
Live adult *Artemia*	*H. americanus*+ *H. gammarus*	70% maximum	Bodega Marine Lab, California	Chang and Conklin, 1993
Live adult *Artemia*	*H. americanus*	60–70%	St Andrews, Canada	Waddy and Aiken, 1998
Live juvenile *Artemia*	*H. americanus*	44–95%	Cutler, Maine	Beal et al., 1998; Beal and Chapman, 2001
Live *Artemia*+ spider crab larvae	*H. gammarus*	75–80% to stages IV and V	France	Nicosia and Lavalli, 1999
Frozen mysids+ chopped mussels	*H. gammarus*	5–30%, mean 14%	Argyll, Scotland	Nicosia and Lavalli, 1999
Frozen *Artemia*	*H. gammarus*	3–5%	Norway	Uglem et al., 1998

% Survival is from hatching to stage IV unless noted otherwise.

ratios were evaluated to determine the most cost-effective diets.

2. Materials and methods

2.1. Facility and animals

Experiments were conducted at the New England Aquarium's Edgerton Research Laboratory in Boston, MA. Animals were maintained in a semi-closed filtered and UV sterilized seawater system (30.5–34‰ salinity, pH 7.84–7.97, NH_4<70 ppm, tested weekly) and were subjected to a daily artificial light cycle (13–11 h LD, wide spectrum fluorescent lighting). Temperatures during the larval periods of both trials were maintained at approximately 18.2 °C to maximize postlarval size (MacKenzie, 1988). Larvae were hatched in this facility from wild-caught ovigerous female American lobsters (*H. americanus*) provided by the Massachusetts State Lobster Hatchery at Martha's Vineyard, MA. In trial 1, larvae were from a 104-mm carapace length (CL) female acquired in July 2002 with the first eggs already hatching. In trial 2, larvae were from an 81-mm CL female that was acquired in September 2002 with embryos 33% developed (estimated by method of Helluy and Beltz, 1991). This female was held at 19–23 °C, prior to being transferred to a hatching tank at 18–19 °C in early December when hatching began. For each trial, larvae were collected from a single day's hatch that occurred near the median larval release day for the clutch. Larvae released by the female the previous night were collected in the morning with a small nylon net from a screened basket in the hatching tank (Waddy and Aiken, 1984). The larvae were counted and 600 strong swimming individuals were selected for the experiment, from a total of 766 (trial 1) and 798 (trial 2) larvae. The experimental larvae were reared in a system of twelve 2-l kreisels (minikreisels) designed for this study based on Hughes et al. (1974). Minikreisels were constructed from 2-l Nalgene polypropylene beakers, with a central standpipe screened with 175-μm nylon mesh to prevent loss of small diet particles to the drains. Water was introduced into the minikreisel through a perforated ring at the base of the standpipe. Fifty larvae were stocked in each minikreisel and three replicate minikreisels were designated per diet per trial.

2.2. Diets and rearing procedures

Nutritional profiles and ingredient lists of available CAR diets were compared to reference diets established for juvenile lobsters (Conklin, 1995) and to profiles for *Artemia*. Particular attention was paid to protein source and content (necessary for adequate growth; Boghen and Castell, 1981), cholesterol (essential for all crustaceans; Teshima, 1997), lipid levels (for energy; Sasaki et al., 1986) and phospholipids (to prevent molt-death syndrome; Teshima, 1997). Only CAR diets containing marine protein, cholesterol and soy lecithin were selected. Particle size and type were also taken into consideration. A particle size of ~800–1200 μ would have been preferable for stage II and III larvae, but available commercial diets in that size range tended to be deficient in one or more essential nutrients. Progression 3, Artemac 5 and Economac 4 were three diets that met the nutritional requirements and included both microencapsulated and microparticulate extruded forms (Table 2).

Kurmaly et al. (1990) suggested that conditioning plays an important role in the feeding strategy of carnivorous larvae, and based on their experiments on European lobster larvae suggested that feeding attractants need to be regularly alternated if the larvae are to continue to ingest microencapsulated diets. Presuming American lobsters might also exhibit this behavior, a daily rotation of three CAR diets was used. Thus, the "rotation diet" consisted of equal portions by weight of Progression 3 in the morning, Artemac 5 at noon and Economac 4 in the afternoon. Diets of frozen adult *Artemia* (F), live *Artemia* nauplii (L) and the CAR rotation diet (R) were tested singly and in paired combinations using a three-way factorial design over the course of two trials. The two best performing treatments from trial 1 (RL and LF) were also repeated in trial 2. Diet treatments of no food and the three food types (RLF) were not conducted. In trial 1, the diets tested included: "R"—rotation diet only, "RL"—50% rotation diet plus 50% live *Artemia* nauplii, "LF"—50% live *Artemia* nauplii plus 50% frozen adult n-3 fatty acid enriched *Artemia* and "F"—frozen adult n-3 fatty acid enriched *Artemia* only. In trial 2, the diets

Table 2
Diet costs and proximate analyses for diets utilized in all larval feeding experiments

Diet	Frozen *Artemia*[a]	Live *Artemia*[b]	Artemac 5[c]	Economac 4[c]	Progression 3[b]
Size	adult	48 h nauplii	500–800 μm	500–800 μm	100–250 μm
Type	*A. salina*, Omega-3 enriched	*A. franciscana*, Select Premium Grade cysts	Micro-encapsulated	Micro-particulate	Micro-encapsulated
Cost (as sold)	$18/kg	$75/454 g	$37.50/kg	$9.50/kg	$38/kg[d]
Cost/kg as fed (dry wt.)	$140 (estimate)	$974	$37.50	$9.50	$38
Proximate analysis[e]					
Protein	5.02% min.		57.0%	57.0%	50% min.
Crude fat/total lipid	1.3% min.		19.0%	13.5%	12% min.
Carbohydrate (by subtraction)	–		12.0%	15.7%	–
Fiber	0.29% max.		–	–	–
Ash	–		5.0%	7.0%	12% max.
Moisture	92.5% max.		7.0%	6.9%	5% max.

[a] San Francisco Bay Brand, 8239 Enterprise Dr., Newark, CA 94560.
[b] Salt Creek, 3528 West 500 South Salt Lake City, UT 84104.
[c] Aquafauna Bio-Marine, P.O. Box 5, Hawthorn, CA 90250.
[d] Diet was provided for free by manufacturer in exchange for a copy of the experimental results.
[e] All values are as given by vendors. Values for the *Artemia* nauplii were not available, as they vary with culture conditions.

tested included: "RF"—50% rotation diet plus 50% frozen adult n-3 fatty acid enriched *Artemia*, "RL"—50% rotation diet plus 50% live *Artemia* nauplii, "LF"—50% live *Artemia* nauplii plus 50% frozen adult n-3 fatty acid enriched *Artemia* and "L"—live *Artemia* nauplii only. For all diets, larvae were fed equal amounts of the appropriate diets by weight, based on published feeding rates for *H. americanus* larvae (Logan and Epifanio, 1978). All larvae received 0.4 μg (dry wt.) diet/day/μg wet body weight. *Artemia* were assumed to be 10% dry weight as fed. Body weights at the different stages were assumed to be those given by Sasaki et al. (1986): 8 mg at stage I, 14 mg at stage II and 25 mg at stage III. At least once a week, the numbers and stages of surviving larvae were assessed in each kreisel in order to adjust feeding rates.

Prior to the morning feeding, uneaten food from the previous day was removed by changing the kreisel standpipe screens and siphoning kreisel bottoms. Food was administered by pipet three times per day (morning, noon and late afternoon). Pre-weighed frozen and dry diets were mixed with seawater just prior to feeding. Live *Artemia* nauplii were strained in a dip net, weighed, resuspended in filtered seawater to a known concentration and fed volumetrically to the larvae. Frequently through the day, a pipet was used to flush small food particles off the standpipe screens and to resuspend frozen *Artemia* off of the minikreisel bottoms.

All CAR diet components (Table 2) were acquired prior to the experiments and used throughout. Two lots of frozen *Artemia* were used, one for the first trial and another for the second trial. Frozen *Artemia* from the first lot were brown and mostly intact. Frozen *Artemia* from the second lot were red, intact and smaller than normal. In the first lot *Artemia* were approximately 9 mm in length, while in the second lot they were approximately 6–7 mm. The live *Artemia* nauplii were 48 h *Artemia franciscana* hatched from decapsulated cysts and harvested daily after 24 h of enrichment with Super Selco. Two lots of cysts were used—one during the first trial and another during the second trial. During trial 1, on days 8, 10, 11, 12, 15 and 19, the 48-h nauplii cultures died for unknown reasons. On those days, larvae were fed 24-h nauplii that had been enriched for 1 h, instead of the usual 48-h nauplii.

At metamorphosis, postlarvae were given a unique identification number and were assessed for abnormalities, which included missing appendages, missing eyes, molted exuvium attached to appendages and intermediate postlarval stages. Postlarvae were blotted dry with a tissue, weighed on a Mettler AB50 digital balance, and then transferred to individual rearing

cups in a sea tray. At this point, they were then switched to a diet of frozen adult *Artemia* supplemented with live *Artemia* nauplii daily and mortalities were noted daily. At approximately 3 months post-metamorphosis, weights were again measured. In trial 2, 30 postlarvae were mistakenly removed from the study population at 1 week post-metamorphosis. Data on those individuals are excluded from the 3-month survivorship and growth analyses.

2.3. Statistical analyses

All data were examined for normality and equal variance prior to analysis. Those data not passing both tests were ranked, and the appropriate parametric statistic was performed on the ranked data. Survival data were tabulated by kreisel (even for the 3-month growing out period) and were analyzed as a two-way ANOVA, where the factors were diet treatment and trial. Pairwise comparisons were examined with Tukey's test. α=0.05 was used in all power calculations. Survival comparisons of larvae with and without abnormalities per kreisel were examined as a paired *t*-test. Observations of individual weight and growth for the 3-month growing out period, calculated as % per day, were analyzed as a two-way ANOVA, where the factors were again diet treatment and trial. Because there were potentially disparate results between some diets conducted in the different trials, and the unbalanced design did not allow for analysis of interactions, a one-way ANOVA was also conducted using the eight independently run diet treatments as factors.

Benefit–cost ratios of the larval diets were calculated as % survivorship/diet unit cost ($/kg$_{dw}$) for survival to metamorphosis and for survival from hatching to 3 months post-metamorphosis. The per kilogram costs as fed (dry weight, excluding labor, storage, shipping and taxes) of the diets used in this investigation at the time of their purchase from commercial retailers were as follows (see also Table 2): the rotation diet (R) was \$28.33/kg$_{dw}$ (equal proportions of Progression 3 \$38/kg$_{dw}$, Artemac 5 \$37.50/kg$_{dw}$ and Economac 4 \$9.50/kg$_{dw}$), frozen *Artemia* (F) was \$140/kg$_{dw}$ and live *Artemia* nauplii (L) was calculated to be \$974/kg$_{dw}$, based on cost of actual yield from New England Aquarium methodology. The larval combination diets (RL, RF and LF) were equal proportions of the two components by dry weight and costs were calculated accordingly. The unit costs of diets for 3-month post-metamorphic juveniles were calculated as means of 3 weeks of the larval diets fed plus 12 weeks of frozen *Artemia*, since the proportion of live *Artemia* nauplii fed daily after metamorphosis was negligible.

3. Results

3.1. Survival

Mortality during the larval period was primarily due to cannibalism (larvae were partially or completely consumed). The number of larvae remaining per kreisel in late stage III did not differ significantly between diet treatments (two-way ANOVA, $F_{5,17}$=2.49, P>0.07, power=0.375) or trials (two-way ANOVA, $F_{1,17}$=3.96, P>0.06, power=0.359). Survival through the metamorphic molt, from late stage III to stage IV ranged from 48.6% in the F and 60.0% in the R treatments to 88.5% in the LF and 88.0% in the RF treatment. There was a four-fold difference in survival from hatching to metamorphosis between diet treatments (Table 3). However, differences were not significant for diets (two-way ANOVA, $F_{5,17}$=2.29, P>0.09, power=0.342) or trials (two-way ANOVA, $F_{1,17}$=0.23, P>0.60).

Table 3
Percent survival of American lobsters during larval (stages I to IV), and postlarval stages (stage IV to 3 months) for both "all" larvae, and those that had no metamorphic abnormalities ("high quality"), as well as the cumulative total (stage I to 3 months). The diets indicate if animals were fed the rotation diet (R), frozen adult *Artemia* (F) or live *Artemia* nauplii (L). Subscript indicates the diet trial. Superscripts denote statistical similarity as tested with one-way ANOVA and paired comparisons with Tukey's test. Values are means±1 S.E.

Diet	Larval	Postlarval (All)	Postlarval (High quality)	Cumulative
R_1	6.00±2.00	58.33±22.05^{a,b}	77.78±22.22	2.667±0.667^{a}
F_1	12.00±7.57	10.26±10.26^{a}	33.33±27.22	2.667±2.667^{a}
L_2	12.00±2.00	64.29±10.71^{a,b}	66.67±8.33	7.500±1.500^{a,b}
LF_1	18.00±4.62	34.30±7.36^{a,b}	55.95±22.62	6.667±2.404^{a,b}
LF_2	15.33±0.67	70.83±6.37^{b}	70.83±6.37	10.778±0.484^{a,b}
RL_1	18.67±1.76	48.99±8.76^{a,b}	54.17±4.17	9.333±2.404^{a,b}
RL_2	24.00±4.62	41.99±14.72^{a,b}	51.59±11.03	8.900±1.412^{a,b}
RF_2	24.67±1.33	50.00±0.00^{a,b}	51.67±11.67	12.333±0.667^{b}

Survivorship from metamorphosis to 3 months of high quality postlarvae, here defined as postlarvae with no noted abnormalities at metamorphosis, was similar for both trials (two-way ANOVA, $F_{1,16}$=0.07, P>0.70) and all diets (two-way ANOVA, $F_{5,16}$=1.02, P>0.40, Table 3). Overall postlarval survival, which included postlarvae with abnormalities, was likewise similar between trials (two-way ANOVA, $F_{1,16}$=1.17, P>0.25) and diets (two-way ANOVA, $F_{5,16}$=1.11, P>0.35). However, survival ranged from a high of 70.83% for postlarvae from the live plus frozen *Artemia* larval diet treatment (LF) in the second trial to a low of 10.26% for the frozen *Artemia* only diet (F). Because of this large difference, these data were also analyzed as a one-way ANOVA. This analysis revealed that the survival of animals fed LF (trial 2) and F (trial 1) was significantly different (one-way ANOVA, $F_{7,16}$=2.56, P<0.05, Tukey's test, q=0.034, P<0.05; Table 3).

Survival from metamorphosis to 3 months was related to postlarval quality at metamorphosis. Postlarvae without abnormalities were more likely to survive 3 months than those with abnormalities (paired t-test, df=14, t=2.865, P=0.012, power=0.71). Between diet treatments, overall survival to 3 months was not related to the mean weight at metamorphosis of the postlarvae produced (two-way ANOVA, $F_{5,184}$=1.987, P>0.08, power=0.341). Combining all diet treatments and trials, animals that survived to 3 months were larger at metamorphosis than animals that did not survive (two-way ANOVA, $F_{1,192}$=6.43, P<0.02, power=0.64). However, for animals that had frozen *Artemia* in the larval diet (F, RF and LF), there was no significant difference in metamorphic weight between postlarvae that did and did not survive for 3 months (paired t-test, df=6, t=0.778, P>0.40). For animals that had not been fed frozen *Artemia* as larvae (R, RL and L), postlarvae that survived to 3 months were significantly larger at metamorphosis than those that did not survive (0.041±0.002 and 0.036±0.003 g, respectively, paired t-test, df=7, t=−4.587, P<0.003).

Cumulative survival (hatch to 3 months) exhibited significant diet treatment effects (one-way ANOVA, $F_{7,16}$=4.125, P<0.01, power=0.809; Table 3) and was similar to the metamorphosis to 3 month data in that animals fed the frozen *Artemia*-only (F) diet had the lowest survival (Table 3). Cumulative survival differed in that the rotation-only (R) diet was equivalent to the F treatment, while the rotation plus frozen *Artemia* combination diet (RF) had the highest overall survival (Tukey's test, q=5.577, P<0.05; Table 3). All other diets were intermediate to and not significantly different from these extremes.

3.2. Growth

Trial and diet both influenced development rate. The larvae took longer to metamorphose to stage IV in trial 1 than in trial 2 (Fig. 1). Within each trial, only the inclusion or absence of live *Artemia* nauplii had an effect. Days to 50% metamorphosis were less in diets including live *Artemia* nauplii (RL, LF and L)

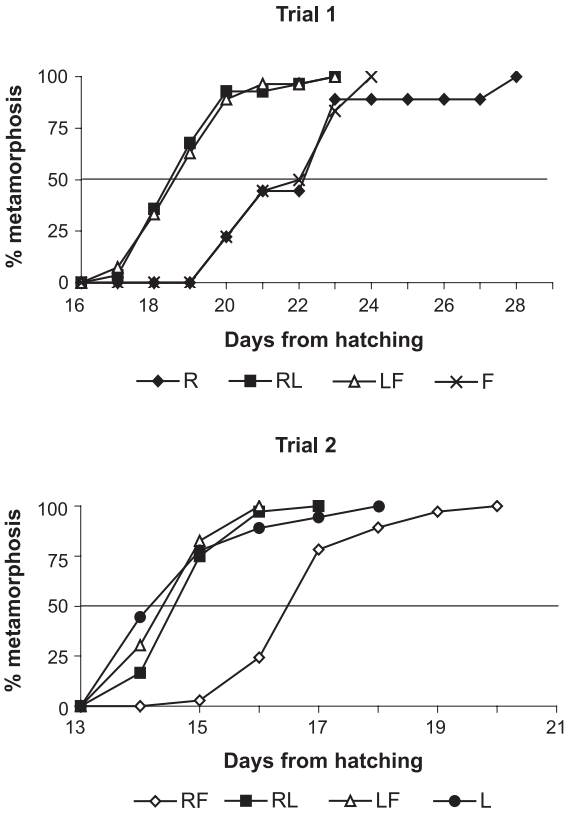

Fig. 1. The cumulative percent of larvae that metamorphosed to stage IV for each diet treatment in trial I (upper) and trial 2 (lower). Days are from hatching. The horizontal line is a 50% reference line. The keys indicate if animals were fed the rotation diet (R), frozen adult *Artemia* (F) or live *Artemia* nauplii (L).

Table 4
Wet weight of American lobsters at metamorphosis and growth rate to 3 months (% bw day^{-1})

Diet	Weight (g)	Growth rate (% bw day^{-1})
R_1	0.0352±0.00182^{a}	6.758±0.661
F_1	0.0384±0.00133^{a,b}	6.782±0.700
L_2	0.0397±0.00128^{a,b,c}	6.973±0.439
LF_1	0.0443±0.00106^{c}	6.107±0.425
LF_2	0.0411±0.00113^{a,b,c}	6.662±0.352
RL_1	0.0417±0.00103^{b,c}	6.654±0.357
RL_2	0.0406±0.00091^{a,b,c}	6.572±0.399
RF_2	0.0397±0.00089^{a,b}	7.179±0.417

The diets indicate if animals were fed the rotation diet (R), frozen adult *Artemia* (F) or live *Artemia* nauplii (L). Subscript indicates the diet trial. Superscripts denote statistical similarity as tested with one-way ANOVA and paired comparisons with Tukey's test. Values are means±1 S.E.

than in diets without live nauplii (R, RF and F; Fig. 1). For all animals in both trials and all diets combined, metamorphosis weight was negatively correlated to metamorphosis date (Pearson product moment correlation, $R=-0.212$, $P<0.002$).

Postlarval weights at metamorphosis varied with diet (two-way ANOVA, $F_{5,189}=4.53$, $P<0.001$, power =0.921) but not trial (two-way ANOVA, $F_{1,189}=3.653$, $P>0.05$, power=0.35). The rotation-only diet (R) resulted in the lowest postlarval weights (Table 4). The heaviest postlarvae were produced by combination diets that included live *Artemia* nauplii (RL and LF; Table 4). Substitution of the CAR rotation for frozen *Artemia* as 50% of the larval diet had no effect on postlarval weight (RF vs. F and RL vs. LF; Table 4). Similarly, replacing 50% of the live *Artemia* nauplii diet with the CAR rotation had no effect on postlarval weight (RL vs. L; Table 4). There was no significant difference in postlarval weights between postlarvae with or without abnormalities (two-way ANOVA, $F_{1,192}=0.68$, $P>0.4$).

All differences in postlarval weights disappeared when the postlarvae were grown out. At 3 months post-metamorphosis, there were no statistical differences in weights between treatments (two-way ANOVA, $F_{7,66}=1.856$, $P>0.09$). Postlarval growth rates (% increase in body weight per day) were likewise similar between larval diet treatments (two-way ANOVA, $F_{5,192}=0.27$, $P>0.9$; Table 4). Growth rates during the growing out period did differ by trial, with the postlarvae growing faster in trial 2 than in trial 1 (6.89±0.34% and 6.54±0.35% per day, respectively, average±1 S.E., two-way ANOVA, $F_{1,69}=5.23$, $P<0.05$, power=0.519).

3.3. Quality of postlarvae

There were diet and trial differences in the proportions of postlarvae with abnormalities at metamorphosis (low quality) vs. postlarvae with no abnormalities (high quality). Abnormalities observed included exuvia adhering to legs after molting, missing claws, missing legs, missing eyes and intermediate stage postlarvae (Fig. 2). There was a higher rate of abnormalities in trial 1 than in trial 2 (ranked data, two-way ANOVA, $F_{1,16}=14.03$, $P<0.005$, power =0.937), yet there were no statistically significant differences between diet treatments

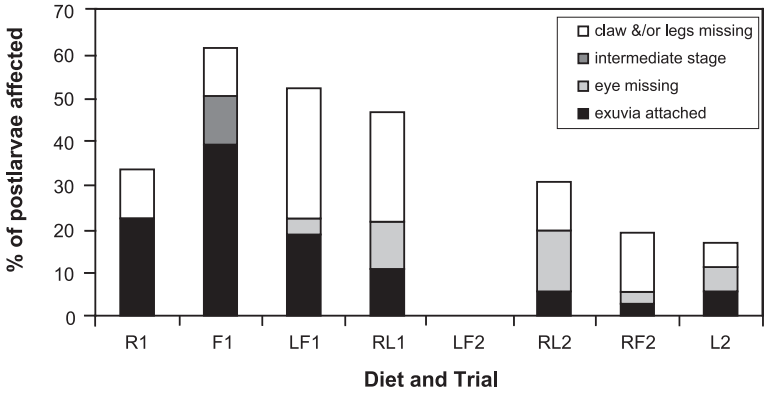

Fig. 2. Incidence of abnormalities observed in postlarvae at metamorphosis. Diets are as identified in Fig. 1. Number after diet indicates trial number.

(two-way ANOVA, $F_{5,17}$=1.32, P>0.3). The lack of statistical significance in diet treatment differences was in part due to large variation in postlarval quality between the trials. More than 50% of the postlarvae from the LF diet in trial 1 had abnormalities, while no postlarvae fed this diet in trial 2 had abnormalities. Also, no larvae survived metamorphosis in one of the three minikreisels fed the frozen *Artemia* only diet (F), thus lessening statistical power. For those postlarvae that were produced, the F treatment had the highest incidence of abnormalities with 11 out of 18 exhibiting an abnormality. The frozen *Artemia* only diet (F) produced the highest incidence of attached exuvia after molting (Fig. 2), and was the only diet in which a stage intermediate to III and IV was generated.

Larvae that received frozen *Artemia* as part or all of their diet were observed to develop a dark hepatopancreas, while larvae not being fed frozen *Artemia* developed a pale, orange hepatopancreas. It did not appear that hepatopancreas color was in any way associated with larval health.

3.4. Physical characteristics of diets and minikreisel performance

The CAR diets were very easy to prepare and administer. They were simply weighed, mixed with water and then dispensed with a pipette. Frozen *Artemia* was also easy to administer but required consistent frozen storage to prevent oxidation. If held at 4 °C for as little as 2 h, thawed *Artemia* turned black. Preparation of live *Artemia* nauplii was labor intensive and time consuming, requiring decapsulation of the cysts, sterilization of the culture vessels and daily harvest.

Progression and Artemac particles retained integrity well for the time they were present in the water (24 h maximum). Economac had similarly good integrity if care was taken to protect the diet from exposure to moisture during storage. Economac appears to be highly hygroscopic. During a separate study, repeated use of a single package in humid weather resulted in the particles becoming slightly sticky, with noted swelling and increased stickiness after 6 h soaking time. This was remedied by dividing newly opened packages into smaller airtight containers stored at 4 °C, to be used sequentially. Small sized diets (Progression and live *Artemia* nauplii) tended to be drawn onto the standpipe screens. Flushing the screens with water by pipette effectively resuspended them. Artemac floated at the water surface for the first 6 h. By the next morning, remaining particles were more neutrally buoyant. Economac was neutrally to negatively buoyant.

Larvae in the R, RL and RF groups were observed feeding on both Artemac and Economac up to 7 h after the feeds were administered, and there was generally only a small amount of uneaten diet to be removed in the mornings. The particle size of the Progression was too small to directly observe ingestion, but within seconds of Progression being administered to the kreisel, larvae responded by vigorous appendage movements similar to that seen with larvae feeding on nauplii. Freshly administered frozen *Artemia* was immediately seized and consumed by larvae, while frozen *Artemia* that had been in the kreisels for two or more hours was often rejected after being seized.

The water circulation pattern in the minikreisels was somewhat suboptimal. While stage I and II larvae were kept suspended and moving in the minikreisels, some stage III larvae tended to settle to the minikreisel bottoms. Similarly, frozen *Artemia* that was not consumed immediately upon administration tended to accumulate on the minikreisel bottoms and standpipe screens. Increasing the water flow rate resulted in the frozen *Artemia* being quickly drawn to the standpipe screens, so instead we opted for periodically flushing with a pipette to resuspend them.

Through trial 1, there was no fouling in any minikreisels. Towards the end of trial 2, there was a slight coating of sticky matter on the surfaces in minikreisels that had the formulated rotation as part of the diet. Water clarity, pH and ammonia levels were within normal parameters throughout both trials.

3.5. Relative benefit–cost

The benefit–cost ratios (Fig. 3) indicated that for survivorship to both stage IV and 3 months post-metamorphosis, the rotation plus frozen *Artemia* combination diet (RF) was the most cost-effective. Even though the rotation-only diet (R) animals had low larval survivorship, the lower cost of the diet made this the second best choice for production of

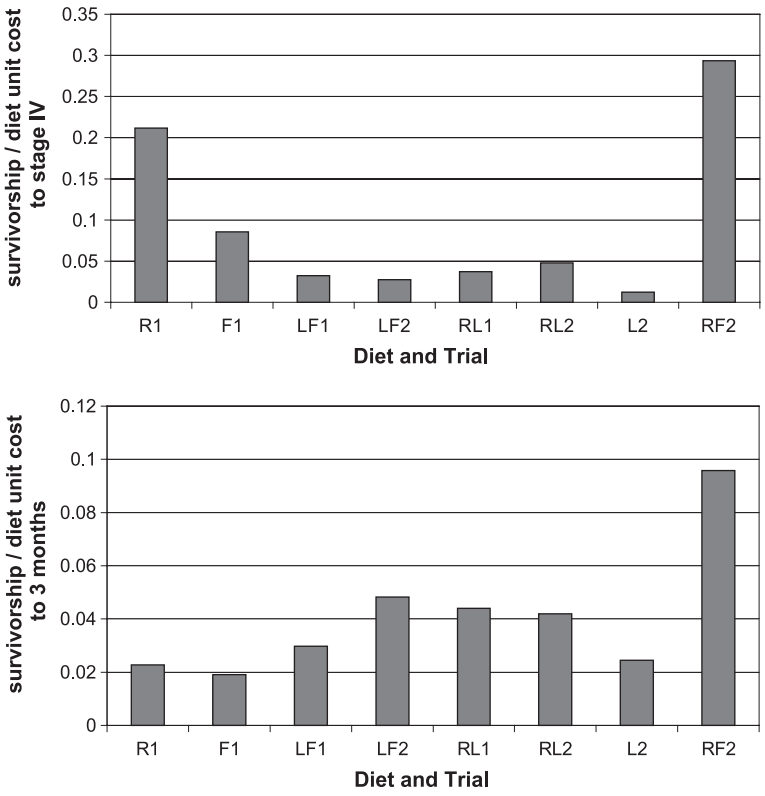

Fig. 3. Benefit (% survivorship) to cost ($/kg$_{dw}$) ratios of diets for lobsters surviving from hatching to metamorphosis (top graph) and those surviving from hatching to 3 months postmetamorphosis (bottom graph). Diets are as identified in Fig. 1.

postlarvae. When animals were grown out, the unit cost of diet was the same for all groups after the 3-week larval period, so survivorship became a more important factor while larval diet cost became somewhat less important.

4. Discussion

This research is the first reported that successfully reared American lobster larvae using artificial feeds. The survival to metamorphosis of animals fed the 100% commercial *Artemia* replacement (CAR) diet in this experiment was 6%, a level similar to that achieved in some hatcheries that feed frozen *Artemia* (Table 1, Uglem et al., 1998). In this study, this survival value was inferior to the other diets. While animals fed only the artificial feeds had relatively low survival, combining that diet with live or frozen *Artemia* increased survival to a point essentially identical to that of the 100% *Artemia* diets. The 50% formulated diets (RF and RL) resulted in the same survivorship as the live plus frozen *Artemia* combination diet (LF), with postlarval production in both trials that were typical of that routinely achieved in this research hatchery. Mortality during the larval period was primarily due to cannibalism, and was not increased by the substitution of 50% of the *Artemia* with the formulated diets. In contrast, the lower survival (6%) to stage IV of animals fed the R diet could be attributed to increased cannibalism.

The frozen *Artemia*-only diet (F) produced a somewhat different pattern of mortality from the other diets. Survival was similar to the combination diets from hatching to late stage III, after which many larvae did not survive metamorphosis. Many of those that did survive exhibited difficulties completing the metamorphic molt (Fig. 2). This group had the highest rate of animals with exuvia adhering to the appendages, and was the only diet in which some molted to

an intermediate stage between III and IV. This pattern of mortality and adhering exuvia was consistent with the results Eagles et al. (1986) achieved when feeding poor quality frozen *Artemia* to American lobster larvae. Adverse conditions can cause a stage III larva to molt to an intermediate stage rather than to the normal stage IV postlarva (Templeman, 1936; Charmantier and Aiken, 1987; Chang and Conklin, 1993). The appearance of intermediate stage larvae and a higher rate of molting difficulties exemplify the inadequacy of frozen *Artemia* as a complete diet. Frozen *Artemia*, particularly from commercial sources, is notorious for being variable in quality (Aiken et al., 1982; Eagles et al., 1986). It is possible that the initial freezing process and/or conditions during shipping and storage lead to degradation of essential phospholipids (Sasaki and Capuzzo, 1984). This problem could apply to other frozen diets as well. Wickins et al. (1995) found that *H. gammarus* larvae reared on a diet of frozen mysids alone had a higher rate of molting problems than larvae fed a diet of frozen mysids supplemented with live *Artemia* nauplii.

Ideally, a healthy or high-quality postlarva is intact after metamorphosis, with no malformations and no remnants of the molted exuvium remaining. In the present study, attached exuvia were more prevalent in the first trial than in the second. Two known causes of molting difficulties that result in the molted exuvium remaining attached to the lobster are epibiotic infestation (Aiken and Waddy, 1995) or inadequate phospholipid levels (Conklin et al., 1980; Bowser and Rosemark, 1981). Here, epibiotic infestation did not appear to be a problem. The only treatments with any signs of container fouling by the end of the larval period were RL and RF in trial 2, which had levels of attached exuvia equal to or lower than L, which had no visible fouling. Thus, the attached exuvia observed here were more likely to have been the result of suboptimal phospholipid levels or quality. There are two sources of phospholipids available to larvae: residual yolk lipids and lipids supplied in the diet. Since the larvae in the two trials originated from two different females, it is possible that egg lipid levels differed between the two females. In order to have larvae hatching in December for trial 2, embryonic development was accelerated by holding the ovigerous female at elevated temperatures. Sasaki et al. (1986) found that such treatment resulted in larvae having more lipid remaining at hatching than larvae that had a normal embryonic development time. There also could have been a difference in the nutritional quality of both the live and frozen *Artemia*. Different lots of both were used for the two trials and the 24-h nauplii used on several days in trial 1 probably had lower lipid levels than the usual 48-h nauplii.

The pale hepatopancreas color of the larvae that had no frozen *Artemia* in their diets (R, RL and L) did not appear to have any impact on the larvae. Harding and Fraser (1999) noted that postlarval pigmentation may be variable, ranging from red and green to blue and yellow even in wild postlarvae, and was not related to larval condition (where condition was measured by lipid storage).

In the present experiment, there were dietary and trial differences observed in larval growth. Larval development rate and postlarval size at metamorphosis may be influenced by many factors. Among these are temperature (Aiken and Waddy, 1986; MacKenzie, 1988), salinity (Templeman, 1936), light intensity (Templeman, 1936; Eagles et al., 1986), photoperiod (Aiken et al., 1981, 1982), season (Aiken et al., 1982), hatching order, or larval size and lipids at hatching (Aiken and Waddy, 1986; MacKenzie, 1988; Wickins et al., 1995; Annis et al., 2003), food quantity (Templeman, 1936; Aiken and Waddy, 1986; Eagles et al., 1986) and diet quality (Eagles et al., 1986; Chang and Conklin, 1993; Wickins et al., 1995). Here, temperature, salinity, light intensity, photoperiod, food quantity and hatching order were constant. Therefore, relevant variables influencing growth may have been hatching season, larval size and lipids at hatching, genetic variation and diet quality.

The animals fed the 100% CAR rotation diet (R) weighed less at metamorphosis than the postlarvae in the other diet groups. Although postlarval weights from larvae fed live nauplii in this experiment were statistically similar to animals fed the CAR rotation plus frozen *Artemia* combination diet (RF), the inclusion of live nauplii in the diet may enhance postlarval weight compared to single diets (R vs. RL and F vs. LF in trial 1). Wickins et al. (1995) also observed that earlier metamorphosing *H. gammarus* postlarvae fed live nauplii were significantly larger than early postlarvae on diets without live nauplii. The inclusion of live nauplii in the diet also appears to

improve larval development rates. In this experiment, animals fed live *Artemia* nauplii metamorphosed earlier on average than those that were not fed this food source, which is also consistent with results that Wickins et al. (1995) obtained. It may be that lipids in the live nauplii contribute to this difference. In stage I, II and III lobster larvae, lipids provide the primary source of energy, being rapidly utilized rather than stored (Sasaki et al., 1986), and therefore need to be continually supplied in the diet. Lipids in frozen foods are subject to degradation by lipases and oxidation (Sasaki and Capuzzo, 1984) and may be of less utility to the larvae. Digestive enzymes in the live nauplii may also contribute to increased larval growth by facilitating digestion of foods in the gut (Kurmaly et al., 1990; Dhont et al., 1993; Kumlu, 1999). As a complete diet, the formulated rotation (R) was suboptimal, producing postlarvae smaller than those from any of the other diets. The rotation diet was able, however, to support normal growth when used to replace 50% of the *Artemia* diet, a result similar to that observed for the mud crab (Genodepa et al., 2004). This suggests that even in the absence of exogenous digestive enzymes from live nauplii, as would be the case in the RF group, the formulated components were digestible and nutritious enough for the larvae to utilize.

Finally, larval dietary conditions were observed to have a prolonged effect into the post-metamorphic period. Postlarvae that had abnormalities, either from prior nutritional deficiency or from cannibalistic encounters with other larvae, were found here to be less likely to survive the first 3 months after metamorphosis. It is unclear whether postlarval size at metamorphosis (here measured as weight) influences subsequent survival. Previous authors have noted that earlier postlarvae to metamorphose are larger and it has been suggested that these animals may be preferable for growing out (Aiken and Waddy, 1995; Wickins et al., 1995). In the present experiment, for all animals combined, weight at metamorphosis was negatively correlated to metamorphosis date, and overall, the mean weight of those that survived 3 months was higher than the mean weight of those that did not. However, this trend may be diet-specific and warrants further investigation. In assessing 4-week survival of grown out *H. gammarus* postlarvae that had been fed larval diets of frozen mysids, either alone or with mussel or *Artemia* supplements, Wickins et al. (1995) found no difference between postlarvae with early or late metamorphosis times.

Conklin et al. (1983) have described the ideal formulated diet, in part, as not requiring frozen storage, and being palatable with good stability in water. The CAR diets tested here met all of those qualifications. The formulated diets were easy to store, prepare and use. Unopened packages were stored at room temperature, and opened ones were stored in a refrigerator, with good retention of quality for over 8 months. The most important factor in preserving the CAR diet quality appeared to be minimizing exposure to moisture, especially for the Economac 4. All three CAR diet components were accepted and ingested by the larvae, even when *Artemia* was also available, and palatability was retained for at least several hours soak time. In communal culture, this can be important. In situations where food is administered a limited number of times per day, cannibalism may increase between feedings if palatable food is unavailable.

Frozen *Artemia* required large amounts of freezer space for storage to prevent a rapid decline in quality and, because of its high water content, it occupied almost 10 times the storage space of the other diets. When freshly administered to the kreisels, it was the most readily accepted of the diets tested. However, palatability decreased rapidly with soak time. The live nauplii required daily husbandry for preparation, and was subject to occasional failure (culture died overnight) or contamination with pathogens such as *Vibrio* if the system was not thoroughly bleached out at least weekly. The nauplii, being alive, retained palatability at all times but were smaller than the preferred prey size for stage II and III lobster larvae.

The minikreisel design was sufficient for this experiment, although reduced water movement on the bottom of the container allowed larger larvae, frozen *Artemia* and Economac to settle on the bottom. While this probably gave the larvae better access to the diets, it also gave them better access to each other, possibly resulting in increased damage or mortality from cannibalism. A better design would be a conical bottomed vessel, with an air-

dispersal ring at the bottom encircling a central standpipe. Vigorous aeration has been demonstrated to be a successful method for generating turbulence in lobster larval culture (Beal and Chapman, 2001), and having the air stream encircling the standpipe helps to prevent diet particles from accumulating on the standpipe screen in a flow-through system (Dhont et al., 1993).

5. Conclusion

The identification of inexpensive, off-the-shelf dry diets that can be directly used in the larval lobster diet is an important milestone in lobster aquaculture. Not only can significant savings be immediately realized in diet purchase, storage and labor, but also hatchery production can become more predictable and reliable. Both the price and nutritional quality of *Artemia* are known to be very variable, while the CAR diets can help to stabilize costs and provide predictable, consistent nutrition. This research demonstrated that, while the CAR rotation diet was inadequate as a complete diet, it could be used as a partial diet to replace either frozen adult *Artemia* or live *Artemia* nauplii. When provided as 50% of a larval American lobster diet, yield was equivalent compared to a 100% *Artemia* diet. Furthermore, postlarvae produced were of equivalent size and quality to those fed *Artemia* only. Postlarval quality proved to be the best predictor of subsequent early juvenile survival. The cost-effectiveness of this diet makes it a tenable solution for feeding larval American lobsters.

Acknowledgments

The authors wish to thank the many interns and volunteers who assisted in the husbandry for this experiment. This research was partially funded by a grant from the Northeast Massachusetts Aquaculture Center. Salt Creek provided the Progression 3 for free in exchange for a copy of the experimental results. The authors declare that they have no financial or other association with any feed manufacturers or suppliers. Two anonymous reviewers provided comments on a draft of this manuscript.

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